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SHORT COMMUNICATIONS

Relatedness by Nucleic Acid Hybridization of New Isolates of Human T-Cell Leukemia–Lymphoma Virus (HTLV) and Demonstration of Provirus in Uncultured Leukemic Blood Cells

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Human T-cell leukemia-lymphoma virus (HTLV) has now been isolated from many different patients with cutaneous T-cell lymphoma and leukemia, as judged by detection of media reverse transcriptase and virus particles and of antigenic determinants related to those of viral structural proteins p24 and p19. Molecular hybridization experiments with HTLV cDNA to viral mRNA or proviral DNA to ascertain the relatedness of four of these new isolates to the first HTLV isolate have been used. By these assays, three appear virtually indistinguishable from the original isolate, HTLV-I(CR), the second U. S. isolate (HTLV-I[MB]), and the Japanese ATLV isolates. Proviral sequences indistinguishable from those of HTLV-I(CR) were also detected in uncultured leukemic blood leukocytes from a patient of Japanese origin with adult T-cell leukemia. These viral isolates thus form a closely related virus group, HTLV-I. In contrast, however, RNA and DNA from one cell line derived from a patient with a T-cell variant of hairy cell leukemia, which expresses media reverse transcriptase and antigenic determinants related to but distinguishable from HTLV p24, did not hybridize substantially with HTLV cDNA. This latter virus appears to represent a second type of HTLV (HTLV-II), related to but substantially different from HTLV-I.

Human T-cell leukemia-lymphoma virus (HTLV), a retrovirus first isolated from T lymphocytes of a U. S. adult with cutaneous T-cell lymphoma (*1*), was shown to be distinct from a wide variety of previously described retroviruses by nucleic acid homology (*2*), protein serology (*3–5*), and the amino acid sequence of the major core protein, p24 (*6*). It was also shown to not be an endogenous virus of humans transmitted through germ lines (*2, 7*). The detection of antibodies to HTLV in a nonblood relative (*8*) and the presence of provirus in some cells of the patient of origin but its absence in others (*2, 7*) strongly suggested viral transmission by infection. Shortly after the first isolation, cultured T cells from a second adult (with T-cell leukemia with cutaneous involvement) were found to be producing a virus indistinguishable from the first HTLV isolate (*9*).

The sporadic detection of antibodies to HTLV in cutaneous T-cell lymphoma-leukemia patients in the United States (*8, 10*) and its nearly ubiquitous presence in related diseases and in some normal persons from Japan (*11–13*) and the Caribbean (*14, 15*) indicated that HTLV-related viruses were widely present in some parts of the world. Workers in Japan confirmed the presence of retrovirus (which they called ATLV) in several cases of adult T-cell leukemia (*16–18*) and the widespread presence of antibodies against (*16*) and proviral DNA related to (*18*) this virus. ATLV, like the second HTLV isolate, is clearly another isolate of HTLV (*19*). Using the presence of antibody as a criterion to select samples from which to attempt virus

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isolation, HTLV-related viruses have since been isolated from 12 other patients (*20, 21*). Four of these isolates were tested in this report for nucleic acid homology to the first two HTLV isolates and to ATLV by molecular hybridization in liquid.

HTLV [^{3}H]cDNA (20×10^6 cpm/μg) was prepared using a random calf thymus DNA primer and hybridized with nuclear DNA or cytoplasmic RNA as described (*2*). Cells were homogenized and fractionated and DNA and RNA prepared from the nucleus and cytoplasm, respectively, by Pronase-SDS digestion and organic solvent extraction. Hybridization was assayed by digestion with S1 nuclease. Cells were cultured in the presence of T-cell growth factor (TCGF) (*22, 23*) as described (*7*) or obtained from the patient by plasmapheresis. T-Cell clone B2 from patient CR was the source of the prototype HTLV (HTLV-I[CR]). Consistent with earlier results, HTLV [^{3}H]cDNA hybridizes to the same extent to cytoplasmic RNA of cultured T cells from the first patient CR (Fig. 1, top panel) and from the second patient MB (Fig. 1, bottom panel), indicating a high degree of sequence homology between the two viruses. An equivalent level of hybridization is observed with RNA from

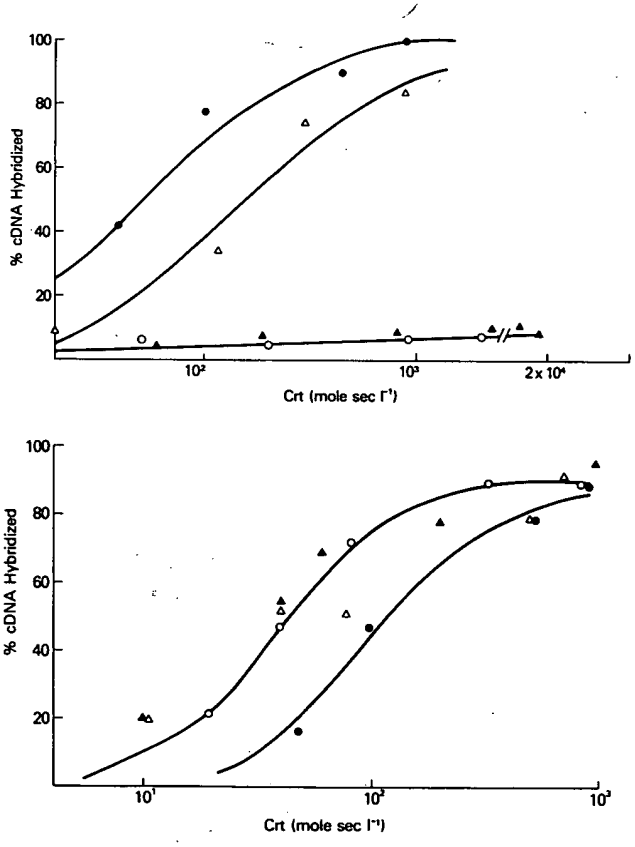

FIG. 1. Hybridization of T-cell mRNA with HTLV-I(CR) [^{3}H]cDNA. HTLV [^{3}H]cDNA was hybridized with cytoplasmic RNA (1–10 μg/μl) in 50% formamide–0.45 *M* NaCl (37°) to the indicated C_rt and the amount of hybridization assayed by S1 nuclease digestion as described (*2*). All values were corrected for $t = 0$ (4–6%) and normalized to 100% hybridization for Clone B2 (actual value was 60% with cytoplasmic RNA). Top panel: HTLV [^{3}H]cDNA was hybridized with RNA from Clone B2 (●), CR-B (○), MO (▲), or MT-2 (△). Bottom panel: HTLV [^{3}H]cDNA was hybridized with RNA from MB (▲), MI (○), MJ (●), or UK (△).

MT-2, a cell line producing ATLV (*16*) (also in agreement with earlier results), indicating that ATLV is highly related to these previous HTLV isolates. No hybridization is detectable with RNA from an Epstein-Barr-virus transformed B-cell line from patient CR (CR-B).

Cytoplasmic RNA was prepared from T-cell lines from four other patients whose sera contained specific antibodies to HTLV core proteins (Gallo *et al.*, submitted) and whose cultured T cells expressed antigenic determinants related to p24 and p19, media reverse transcriptase, and virus particles (*20, 21*). These included a patient with a cutaneous T-cell lymphoma (mycosis fungoides) from Boston (MJ), one with a peripheral T-cell lymphoma from Israel (UK), one of Caribbean origin (MI) with adult T-cell leukemia (*14*), and one from Seattle with a T-cell variant of hairy cell leukemia (MO) (*24*). As shown in Fig. 1, bottom panel, RNA from the first three T-cell lines hybridized to HTLV [^{3}H]cDNA to the same extent as did both the homologous RNA (Fig. 1, top panel) and RNA from cells producing the second HTLV isolate MB (Fig. 1, bottom panel). The $C_rt_{1/2}$ values ranged from about 30 to 200, indicative of a viral RNA content of approximately 0.2–0.05% by weight, using 0.14 as the $C_rt_{1/2}$ of pure viral genomic RNA (*2*). In contrast, RNA from the MO cell line, which produces an HTLV-related virus (*20*), did not hybridize significantly with HTLV cDNA, even at a C_rt of 20,000. Moreover, when the stringency of hybrid formation was decreased (from 37 to 22°) and that of the S1 nuclease digestion was also decreased (from 43 to 37° and from 0.45 to 0.65 *M* NaCl), the results were essentially identical (not shown). No significant hybridization was observed when total cell RNA or poly(A)-selected RNA was used instead of total cytoplasmic RNA. Viral RNA bands are clearly visible on Northern blots for all HTLV isolates except MO using stringent washing procedures, and with MO only if the stringency is lowered (to 5× SSC at room temperature) (unpublished results with G. Franchini). These data indicate that the MO viral sequences differ substantially from those of the prototype HTLV as well as the other tested HTLV isolates, and are consistent with data which show that the p24 expressed by the T cells of MJ, UK, and MI is indistinguishable from that of HTLV-I(CR) by competition radioimmunoassay, while the p24 from MO differs substantially (*20*). The apparent low degree of homology between the viral RNA of most strains of HTLV and the HTLV produced by the MO line is thus compatible with the p24 data, and these data taken together indicate that the MO virus is substantially different from the prototype HTLV, which we call HTLV-I.

Nuclear DNA from several T-cell samples was also tested for HTLV-related sequences. As shown previously (*7*), HTLV cDNA hybridized to DNA from clone B2 with a corrected $C_0t_{1/2}$ of 500–600 (Fig. 2), but did not hybridize to DNA from normal human uninfected cells or from the human promyelocytic leukemia cell line HL-60 (*25*). We had previously demonstrated the presence of proviral sequences in uncultured fresh blood cells from patient MB, from whom the second HTLV isolate was obtained (*9*). DNA from uncultured fresh peripheral blood leukocytes from an HTLV antibody-positive patient of Japanese origin (SD) with chronic lymphocyte T-cell leukemia hybridized HTLV cDNA to the same extent as clone B2 DNA, although with a higher $C_0t_{1/2}$ (~3000) (Fig. 2). This $C_0t_{1/2}$ is consistent with the presence of one copy of HTLV provirus per one to two haploid genomes in this leukemic blood sample. DNA from the MO line hybridized only very slightly to HTLV cDNA, confirming the results obtained with MO cytoplasmic RNA. As is true for Northern blots, viral bands with all HTLV isolates but MO are visible on Southern blots washed with high stringency (1× SSC, 65°). With MO DNA, such bands can be detected only by lowering the stringency of washing (3× SSC, 65°) (unpublished results with E. Gelmann).

From the above data it appears that the HTLV isolates from UK, MK, and MI (*21*), the original isolates from CR (*1*) and MB (*9*), and the ATLV isolates subsequently made in Japan (16–18) belong to a closely

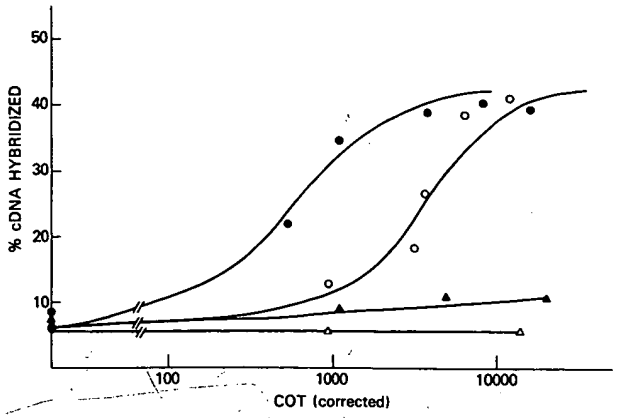

FIG. 2. Hybridization of DNA from human leukemic mature T-cell lines and from uncultured leukemic blood T cells with HTLV-I(CR) [^{3}H]cDNA. Nuclear DNA (600 μg per 500 cpm [^{3}H]cDNA) was hybridized to the indicated corrected C_0t in 0.6 *M* NaCl (65°) and the amount of hybridization assayed by S1 nuclease digestion as described (*2*). DNA was from Clone B2 (●), HL60 (△), MO (▲), and SD, uncultured fresh peripheral blood leukemic leukocytes from a patient with adult T-cell leukemia (○).

related group of viruses (which we call HTLV-I). In contrast, HTLV from MO, as suggested from results with the viral core protein p24 (*20*), appears to differ substantially from this group, and we have tentatively designated it HTLV-II. More detailed comparisons of these viruses by molecular cloning and sequence analyses are currently in progress.

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